

What is claimed is:

1. A probe comprising at least one and preferably all of the following as operably linked components:

- a) a first pair of nucleic acid sequences consisting of a first object sequence and a first complement sequence, said first object and first complement sequences each independently having about 3 to about 150 nucleotides, being substantially complementary to each other, and forming a first hybridized duplex;
- b) at least one recognition element conjugated to at least one of said first object and first complement sequences, said recognition element specifically interacting with at least one target agent; and
- c) optionally, at least one detectable label, said detectable label producing a characteristic signal whose level is a function of the amount of said first hybridized duplex,

wherein in the presence of said target agent, said interaction of said target agent with said recognition element alters the amount of said first hybridized duplex compared to that in the absence of said target agent, altering said characteristic signal.

2. The probe of claim 1 further comprising a second pair of competing nucleic acid sequences consisting of a second object sequence and a second complement sequence,

said second object and second complement sequences each independently having about 3 to about 150 nucleotides, being substantially complementary to each other, and forming a second hybridized duplex; and

said first and second object sequences being contained in an object sequence and having an overlapping region consisting of at least one nucleotide,

wherein in the presence of said target agent, said interaction of said target agent with said recognition element causes decrease in the amount of one of said first and second hybridized duplexes and increase in the amount of the other hybridized duplex compared to those in the absence of said target agent, altering said characteristic signal.

3. A probe according to claim 2, wherein said recognition element is conjugated to said first object sequence excluding said overlapping region or the first complement sequence.
4. A probe according to any of claims 1 and 2, wherein said object and complement sequences  
5 are selected from the group consisting of DNA, RNA, PNA and mixtures of DNA and RNA.
5. An affinity probe according to claim 1, wherein said recognition element is a probe ligand, and said target agent is a receptor agent that can specifically bind to said probe ligand.
- 10 6. An affinity probe according to claim 5, wherein the melting temperature of said first hybridized duplex decreases by at least 1°C upon binding of said receptor agent to said probe ligand.
7. An affinity probe according to claim 5, wherein said probe ligand is covalently linked to said  
15 first object or first complement sequence by at least one coupling elements.
8. An affinity probe according to claim 7, wherein said coupling element is selected from the group consisting of chemical bonds, divalent atoms, divalent chemical moieties, and multivalent chemical moieties.
- 20 9. An affinity probe according to claim 5, wherein said probe ligand is selected from the group consisting of chemical ligands, biochemical ligands, antigens, antibodies, antibody fragments, enzymes, substrates of enzymes, substrates or inhibitors of enzymes, hormones, antibiotics, narcotics, toxins, polypeptides, proteins, protein fragments, targeting sequences, transit peptides,  
25 glycoproteins, lipids, phospholipids, polysaccharides, carbohydrates, nucleic acids, and peptide nucleic acids.
10. A cleavage probe according to claim 1, wherein said recognition element is a probe substrate comprising a destabilizing agent and at least one cleavage site, and said target agent is a reaction-  
30 inducing agent that can specifically cleave said cleavage site, wherein a first end of said cleavage

site is conjugated to said destabilizing agent and a second end of said cleavage site is covalently linked to said first object or first complement sequence by at least one coupling element.

11. A cleavage probe according to claim 10, wherein the melting temperature of said first hybridized duplex increases by at least 1°C upon cleavage of said cleavage site by said reaction inducing agent.

12. A cleavage probe according to claim 10, wherein said coupling element is selected from the group consisting of chemical bonds, divalent atoms, divalent chemical moieties, and multivalent chemical moieties.

13. A cleavage probe according to claim 10, wherein said destabilizing agent is a protein or a protein complex covalently or non-covalently linked to the first end of said cleavage site by a linker.

14. A cleavage probe according to claim 13, wherein said destabilizing agent is a streptavidin or its derivative bound to a biotin, wherein the biotin is covalently linked to the first end of said cleavage site by a linker.

15. A cleavage probe according to claim 13, wherein said linker is selected from the group consisting of chemical bonds, divalent atoms, divalent chemical moieties, and multivalent chemical moieties.

16. A cleavage probe according to claim 10, wherein said cleavage site is specifically cleaved by a protease, an endonuclease, a lipase, or a glycosidase.

17. A cleavage probe according to claim 16, wherein said cleavage site is specifically cleaved by heparanase-1.

18. A cleavage probe according to claim 16, wherein said cleavage site is specifically cleaved by a mammalian or viral protease.

19. A cleavage probe according to claim 16, wherein said cleavage site is specifically cleaved by a protease associated with a human pathogen.
- 5 20. A cleavage probe according to claim 19, wherein the protease is expressed by a cytomegalovirus (CMV); herpes simplex virus (HSV); hepatitis virus; a plasmodium, human immunodeficiency virus (HIV), Kaposi's sarcoma-associated herpes virus (KSHV), yellow fever virus, flavivirus, or rhinovirus.
- 10 21. A cleavage probe according to claim 18, wherein the protease is a serine-type, cystein-type, or aspartate-type protease.
22. A cleavage probe according to claim 20, wherein the plasmodium is *P. falciparum* and the protease is one of plasmepsin I and plasmepsin II.
- 15 23. A cleavage probe according to claim 20, wherein said cleavage site is specifically cleaved by a maturational protease of HSV.
24. A cleavage probe according to claim 20, wherein the hepatitis virus is type C.
- 20 25. A cleavage probe according to claim 19, wherein the human pathogen is yeast, bacterium, fungi, nematode, virus, or protozoa.
26. A cleavage probe according to claim 18, wherein said cleavage site is specifically cleaved by a mammalian protease associated with blood coagulation, apoptosis, Alzheimer's disease or the extracellular matrix.
- 25 27. A Type I coupling probe according to claim 1, wherein said recognition element is a probe substrate comprising at least one reaction site covalently linked to said first object or first complement sequence by at least one coupling element, and the target agent is a reaction-inducing agent that can specifically conjugate a destabilizing agent to said reaction site.
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28. A Type I coupling probe according to claim 27, wherein the melting temperature of said first hybridized duplex decreases by at least 1°C upon conjugation of said destabilizing agent by said reaction inducing agent.

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29. A Type I coupling probe according to claim 27, wherein said coupling element is selected from the group consisting of chemical bonds, divalent atoms, divalent chemical moieties, and multivalent chemical moieties.

10 30. A Type I coupling probe according to claim 27, wherein said destabilizing agent is a protein or a protein complex having at least one reaction group that can be covalently linked to said reaction site in the presence of said reaction inducing agent.

15 31. A Type I coupling probe according to claim 30, wherein said destabilizing agent is a streptavidin bound to a modified biotin that has at least one said reaction group.

32. A Type I coupling probe according to claim 27, wherein said reaction site is specifically conjugated to said destabilizing agent by a ligase.

20 33. A Type I coupling probe according to claim 32, wherein said ligase is selected from the group consisting of polynucleotide ligases, aminoacyl tRNA ligases, and biotin protein ligases.

25 34. A Type II coupling probe according to claim 1, wherein said recognition element is a probe substrate comprising at least one reaction site covalently linked to said first object or first complement sequence by at least one coupling element, and the target agent is a reaction-inducing agent that can specifically convert said reaction site to a conjugation site to which a destabilizing agent can be conjugated.

30 35. A Type II coupling probe according to claim 34, wherein the melting temperature of said first hybridized duplex decreases by at least 1°C upon conjugation of said destabilizing agent by said reaction inducing agent.

36. A Type II coupling probe according to claim 34, wherein said coupling element is selected from the group consisting of chemical bonds, divalent atoms, divalent chemical moieties, and multivalent chemical moieties.

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37. A Type II coupling probe according to claim 34, wherein said destabilizing agent is a protein or a protein complex that can specifically bind to said conjugation site.

38. A Type II coupling probe according to claim 34, wherein said destabilizing agent is a protein or a protein complex having at least one reaction group that can be covalently linked to said conjugation site either spontaneously or in the presence of a coupling reagent.

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39. A Type II coupling probe according to claim 38, wherein said destabilizing agent is a streptavidin or its derivative bound to a modified biotin that has at least one said reaction group.

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40. A Type II coupling probe according to claim 34, wherein said reaction site is specifically converted by a transferase.

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41. A Type II coupling probe according to claim 40, wherein said transferase is selected from the group consisting of kinases and acetyl-CoA transferases.

42. A Type II coupling probe according to claim 41, wherein said kinase is a protein kinase selected from the group consisting of c-AMP dependent protein kinase (PKA), casein kinase I (CKI), casein kinase II (CKII), glycogen synthase kinase 3 (GSK-3), cdc2 protein kinase, calmodulin-dependent protein kinase II (CaMK II), insulin receptor (INSR), mitogen-activated protein kinase (MAPK), cGMP-dependent protein kinase (cGPK), phosphorylase kinase (PhK), protein kinase C (PKC), p34 cdc2 protein kinase, meiosis-activated myelin basic protein kinase (p44 mpk), smooth muscle myosin light chain kinase, epidermal growth factor receptor kinase (EGF-RK), and protein tyrosine kinase pp60c-src (PTK).

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43. A Type II coupling probe according to claim 34, wherein said reaction site is a site specific to a kinase, and said conjugation site includes a phosphate attached by the kinase.

44. A Type II coupling probe according to claim 43, wherein said destabilizing agent is an antibody or a receptor that can specifically bind to said conjugation site having the phosphate attached by the kinase.

45. A Type II coupling probe according to claim 43, wherein said phosphate is a thiophosphate and said destabilizing agent is a streptavidin or its derivative bound to a modified biotin that has at least one reaction group such as iodoacetyl or thiol group that can be covalently linked to said conjugation site in the presence of a coupling reagent.

46. A Type II coupling probe according to claim 43, wherein said destabilizing agent is a nanoparticle, a microparticle, a bead or a membrane made of synthetic polymers or the like containing dicataionic, tricataionic or polycataionic metal ions, that can specifically bind to said conjugation site having the phosphate group.

47. A Type II(-) coupling probe according to claim 1, wherein said recognition element is a probe substrate comprising at least one reaction site covalently linked to said first object or first complement sequence by at least one coupling element, and the target agent is a reaction-inducing agent that can specifically convert said reaction site that is a conjugation site to which a destabilizing agent can be conjugated, to a non-conjugatable site.

48. A Type II(-) coupling probe according to claim 47, wherein the melting temperature of said first hybridized duplex decreases by at least 1°C upon conjugation of said destabilizing agent.

49. A Type II(-) coupling probe according to claim 47, wherein said coupling element is selected from the group consisting of chemical bonds, divalent atoms, divalent chemical moieties, and multivalent chemical moieties.

50. A Type II(-) coupling probe according to claim 47, wherein said destabilizing agent is a protein or a protein complex that can specifically bind to said reaction site.

51. A Type II(-) coupling probe according to claim 47, wherein said destabilizing agent is a protein or a protein complex having at least one reaction group that can be covalently linked to said reaction site either spontaneously or in the presence of a coupling reagent.

52. A Type II(-) coupling probe according to claim 51, wherein said destabilizing agent is a streptavidin or its derivative bound to a modified biotin that has at least one said reaction group.

53. A Type II(-) coupling probe according to claim 47, wherein said reaction site is a site having a phosphate group that can be specifically de-phosphorylated by a phosphatase.

54. A Type II(-) coupling probe according to claim 53, wherein said phosphatase is selected from phosphoprotein phosphatases such as protein tyrosine phosphatases, dual specificity phosphatases and protein Ser/Thr phosphatases; phospholipids phosphatases such as phosphatidylinositol-3,4-bisphosphate 4-phosphatase, phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase, SH2 domain-containing inositol phosphatase (SHIP) and membrane-associated phospholipid phosphatase; and polynucleotide phosphatases such as polynucleotide 3'-phosphatase and polynucleotide 5'-phosphatase.

55. A Type II(-) coupling probe according to claim 53, wherein said destabilizing agent is an antibody or a receptor that can specifically bind to said reaction site having the phosphate group.

56. A Type II(-) coupling probe according to claim 53, wherein said phosphate is a thiophosphate and said destabilizing agent is a streptavidin or its derivative bound to a modified biotin that has at least one reaction group such as iodoacetyl or thiol group that can be covalently linked to said reaction site in the presence of a coupling reagent.

57. A Type II(-) coupling probe according to claim 53, wherein said destabilizing agent is a nanoparticle, a microparticle, a bead or a membrane made of synthetic polymers or the like



containing dicataionic, tricataionic or polycataionic metal ions, that can specifically bind to said reaction site having the phosphate group.

58. An affinity probe according to claim 2, wherein said first hybridized duplex is preferentially  
5 formed compared to said second hybridized duplex in the absence of said target agent.

59. An affinity probe according to claim 58, wherein the melting temperature of said first  
hybridized duplex is at least 1°C higher than the melting temperature of said second hybridized  
duplex in the absence of said target agent.

10 60. An affinity probe according to claim 58, wherein said second hybridized duplex is  
preferentially formed compared to said first hybridized duplex in the presence of an excess of  
said target agent.

15 61. An affinity probe according to claim 60, wherein the melting temperature of said first  
hybridized duplex is at least 1°C lower than the melting temperature of said second hybridized  
duplex in the presence of an excess of said target agent.

20 62. A cleavage probe according to claim 2, wherein said second hybridized duplex is  
preferentially formed compared to said first hybridized duplex in the absence of said target  
agent.

25 63. A cleavage probe according to claim 62, wherein the melting temperature of said first  
hybridized duplex is at least 1°C lower than the melting temperature of said second hybridized  
duplex in the absence of said target agent.

64. A cleavage probe according to claim 62, wherein said first hybridized duplex is preferentially  
formed compared to said second hybridized duplex in the presence of an excess of said target  
agent.

65. A cleavage probe according to claim 64, wherein the melting temperature of said first hybridized duplex is at least 1°C higher than the melting temperature of said second hybridized duplex in the presence of an excess of said target agent.

5 66. A Type I coupling probe according to claim 2, wherein said first hybridized duplex is preferentially formed compared to said second hybridized duplex in the absence of said target agent.

10 67. A Type I coupling probe according to claim 66, wherein the melting temperature of said first hybridized duplex is at least 1°C higher than the melting temperature of said second hybridized duplex in the absence of said target agent.

15 68. A Type I coupling probe according to claim 66, wherein said second hybridized duplex is preferentially formed compared to said first hybridized duplex in the presence of an excess of said target agent.

20 69. A Type I coupling probe according to claim 68, wherein the melting temperature of said first hybridized duplex is at least 1°C lower than the melting temperature of said second hybridized duplex in the presence of an excess of said target agent.

70. A Type II coupling probe according to claim 2, wherein said first hybridized duplex is preferentially formed compared to said second hybridized duplex in the absence of said target agent.

25 71. A Type II coupling probe according to claim 70, wherein the melting temperature of said first hybridized duplex is at least 1°C higher than the melting temperature of said second hybridized duplex in the absence of said target agent.

30 72. A Type II coupling probe according to claim 70, wherein said second hybridized duplex is preferentially formed compared to said first hybridized duplex in the presence of an excess of said target agent.

73. A Type II coupling probe according to claim 72, wherein the melting temperature of said first hybridized duplex is at least 1°C lower than the melting temperature of said second hybridized duplex in the presence of an excess of said target agent.

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74. A Type II(-) coupling probe according to claim 2, wherein said second hybridized duplex is preferentially formed compared to said first hybridized duplex in the absence of said target agent.

10 75. A Type II(-) coupling probe according to claim 74, wherein the melting temperature of said first hybridized duplex is at least 1°C lower than the melting temperature of said second hybridized duplex in the absence of said target agent.

15 76. A Type II(-) coupling probe according to claim 74, wherein said first hybridized duplex is preferentially formed compared to said second hybridized duplex in the presence of an excess of said target agent.

20 77. A Type II(-) coupling probe according to claim 76, wherein the melting temperature of said first hybridized duplex is at least 1°C higher than the melting temperature of said second hybridized duplex in the presence of an excess of said target agent.

25 78. A probe according to claim 1, wherein said detectable label is a non-interactive label selected from the group consisting of fluorescers, luminescers, radioisotopes, enzymes, antibodies, antigens, and electrochemical labels.

79. A probe according to claim 78, wherein said probe is a bimolecular probe consisting of a first molecule comprising said first object sequence and a second molecule comprising said first complement sequence, wherein said at least one detectable label is covalently linked to at least one of said first and second molecules.

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80. A probe according to claim 79, wherein said first molecule is immobilized to a support and said at least one detectable label is covalently linked to said second molecule.
81. A probe according to claim 79, wherein said second molecule is immobilized to a support  
5 and said at least one detectable label is covalently linked to said first molecule.
82. A probe according to claim 1, wherein said detectable label is an intercalating dye that can preferentially bind to double-stranded nucleic acids.
- 10 83. A probe according to claim 82, wherein said probe comprises a first molecule comprising said first object sequence and a second molecule comprising said first complement sequence.
84. A probe according to claim 83, wherein said first or second molecule is immobilized to a support.  
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85. A probe according to claim 82, wherein said first object and first complement sequences are covalently linked by a loop moiety.
86. A probe according to claim 85, wherein said loop moiety connects any of 5' or 3' terminus of  
20 said first object sequence to any of 5' or 3' terminus of said first complement sequence.
87. A probe according to claim 85, wherein said loop moiety is a nucleic acid sequence having about 4 to about 100 nucleotides.
- 25 88. A probe according to claim 87, wherein said first object sequence, said loop moiety, and said first complement sequence are covalently linked in sequence in a 5' to 3' or 3' to 5' direction.
89. A probe according to claim 85 immobilized to a support.
- 30 90. A probe according to claim 1, wherein said at least one detectable label comprises an interactive label pair comprising a first label moiety conjugated to said first object sequence and

a second label moiety conjugated to said first complement sequence, said first and second label moieties interacting when said first hybridized duplex is formed.

91. A probe according to claim 90, wherein said interactive label pair is a fluorescer and a quencher, wherein the interaction between said interactive label pair causes change in the fluorescence of said probe, said change in the fluorescence being selected from the group consisting of intensity decrease, lifetime decrease, polarization change, and wavelength change.

92. A probe according to claim 90, wherein said probe is a bimolecular probe consisting of a first molecule comprising said first object sequence including said first label moiety, and a second molecule comprising said first complement sequence including said second label moiety.

93. A probe according to claim 92, wherein said first or second molecule is immobilized to a support.

94. A probe according to claim 90, wherein said probe is a unimolecular probe, wherein said first object and first complement sequences are covalently linked by a loop moiety.

95. A probe according to claim 94, wherein said loop moiety connects any of 5' or 3' terminus of said first object sequence to any of 5' or 3' terminus of said first complement sequence.

96. A probe according to claim 94, wherein said loop moiety is a nucleic acid sequence having about 4 to about 100 nucleotides.

97. A probe according to claim 96, wherein said first object sequence, said loop moiety, and said first complement sequence are covalently linked in sequence in a 5' to 3' or 3' to 5' direction.

98. A probe according to claim 94 immobilized to a support.

99. A probe according to claim 1, wherein said probe is a unimolecular probe, wherein said first object and first complement sequences are covalently linked by a loop moiety and said at least

one detectable label comprises an interactive label pair comprising first and second label moieties conjugated to different positions of said unimolecular probe, said first and second label moieties interacting when said first hybridized duplex is formed.

5     100. A probe according to claim 99, wherein said loop moiety connects any of 5' or 3' terminus of said first object sequence to any of 5' or 3' terminus of said first complement sequence.

101. A probe according to claim 99, wherein said loop moiety is a nucleic acid sequence having about 4 to about 100 nucleotides.

10     102. A probe according to claim 101, wherein said first object sequence, said loop moiety, and said first complement sequence are covalently linked in sequence in a 5' to 3' or 3' to 5' direction.

15     103. A probe according to claim 99, wherein said interactive label pair is a fluorescer and a quencher, wherein the interaction between said interactive label pair causes change in the fluorescence of said probe, said change in the fluorescence being selected from the group consisting of intensity decrease, lifetime decrease, polarization change, and wavelength change.

20     104. A probe according to claim 99, wherein at least one of said first and second label moieties is covalently linked to said loop moiety.

105. A probe according to claim 99 immobilized to a support.

25     106. A probe according to claim 1, wherein said probe further comprises a pair of nucleic acid arm sequences consisting of a 5' arm sequence covalently linked to 5' terminus of said first object sequence and a 3' arm sequence covalently linked to 3' terminus of said first object sequence, said pair of arm sequences forming a stem duplex having about 3 to about 35 complementary base pairs when said first object sequence is not hybridized to said first complement sequence; and wherein said at least one detectable label comprises an interactive  
30     label pair comprising a first label moiety conjugated to said 5' arm sequence and a second label

moiety conjugated to said 3' arm sequence, said first and second label moieties interacting when said stem duplex is formed.

107. A probe according to claim 1, wherein said probe further comprises a pair of nucleic acid arm sequences consisting of a 5' arm sequence covalently linked to 5' terminus of said first complement sequence and a 3' arm sequence covalently linked to 3' terminus of said first complement sequence, said pair of arm sequences forming a stem duplex having about 3 to about 35 complementary base pairs when said first object sequence is not hybridized to said first complement sequence; and wherein said at least one detectable label comprises an interactive label pair comprising a first label moiety conjugated to said 5' arm sequence and a second label moiety conjugated to said 3' arm sequence, said first and second label moieties interacting when said stem duplex is formed.

108. A probe according to claim 1, wherein said probe further comprises two pairs of nucleic acid arm sequences, a first arm pair consisting of a first 5' arm sequence covalently linked to 5' terminus of said first object sequence and a first 3' arm sequence covalently linked to 3' terminus of said first object sequence and a second arm pair consisting of a second 5' arm sequence covalently linked to 5' terminus of said first complement sequence and a second 3' arm sequence covalently linked to 3' terminus of said first complement sequence, said first arm pair forming a first stem duplex having about 3 to about 35 complementary base pairs and said second arm pair forming a second stem duplex having about 3 to about 35 complementary base pairs when said first object sequence is not hybridized to said first complement sequence; and wherein said at least one detectable label comprises two interactive label pairs, a first label pair comprising a first label moiety conjugated to said first 5' arm sequence and a second label moiety conjugated to said first 3' arm sequence and a second label pair comprising a third label moiety conjugated to said second 5' arm sequence and a fourth label moiety conjugated to said second 3' arm sequence, said first label pair interacting when said first stem duplex is formed and said second label pair interacting when said second stem duplex is formed.

109. A probe according to any of claims 106-108, wherein said interactive label pair is a fluorescer and a quencher, wherein the interaction between said interactive label pair causes

change in the fluorescence of said probe, said change in the fluorescence being selected from the group consisting of intensity decrease, lifetime decrease, polarization change, and wavelength change.

5 110. A probe according to any of claims 106-108, wherein said probe is a bimolecular probe consisting of a first molecule comprising said first object sequence and a second molecule comprising said first complement sequence.

10 111. A probe according to claim 110, wherein said first or second molecule is immobilized to a support.

15 112. A probe according to any of claims 106-108, wherein said probe is a unimolecular probe, wherein a first unit containing at least said first object sequence and a second unit containing at least said first complement sequence are covalently linked by a loop moiety.

113. A probe according to claim 112, wherein said loop moiety connects any of 5' or 3' terminus of said first object sequence to any of 5' or 3' terminus of said first complement sequence.

20 114. A probe according to claim 112, wherein said loop moiety is a nucleic acid sequence having about 4 to about 100 nucleotides.

25 115. A probe according to claim 114, wherein said first object sequence, said loop moiety, and said first complement sequence are covalently linked in sequence in a 5' to 3' or 3' to 5' direction.

116. A probe according to claim 112 immobilized to a support.

30 117. A probe according to claim 2, wherein said detectable label is a non-interactive label selected from the group consisting of fluorescers, luminescers, radioisotopes, enzymes, antibodies, antigens, and electrochemical labels.



118. A probe according to claim 117, wherein said probe is a trimolecular probe consisting of a first molecule comprising said object sequence, a second molecule comprising said first complement sequence, and a third molecule comprising said second complement sequence, wherein said at least one detectable label is covalently linked to at least one of said first, second, and third molecules.

119. A probe according to claim 118, wherein said at least one detectable label is covalently linked to at least one of said second and third molecules, wherein if both molecules are labeled, each molecule has a different label.

120. A probe according to claim 119, wherein said first molecule is immobilized to a support.

121. A probe according to claim 118, wherein one of said second or third molecule is immobilized to a support and said at least one detectable label is covalently linked to said first molecule.

122. A probe according to claim 117, wherein said probe is a bimolecular probe consisting of a first molecule comprising said object sequence and one of said first and second complement sequences, and a second molecule containing a remaining sequence, wherein the two sequences contained in said first molecule are covalently linked by a loop moiety and said at least one detectable label is covalently linked to at least one of said first and second molecules.

123. A probe according to claim 122, wherein said loop moiety connects any of 5' or 3' terminus of a first sequence to any of 5' or 3' terminus of a second sequence.

124. A probe according to claim 122, wherein said loop moiety is a nucleic acid sequence having about 4 to about 100 nucleotides.

125. A probe according to claim 124, wherein a first sequence, said loop moiety, and a second sequence are covalently linked in sequence in a 5' to 3' or 3' to 5' direction.

126. A probe according to claim 122, wherein said first molecule is immobilized to a support and said at least one detectable label is covalently linked to said second molecule.

127. A probe according to claim 122, wherein said second molecule is immobilized to a support  
5 and said at least one detectable label is covalently linked to said first molecule.

128. A probe according to claim 2, wherein said detectable label is an intercalating dye that can  
10 preferentially bind to double-stranded nucleic acids.

129. A probe according to claim 128, wherein said probe comprises a first molecule comprising  
said object sequence, a second molecule comprising said first complement sequence, and a third  
molecule comprising said second complement sequence.

130. A probe according to claim 129, wherein said second or third molecule is immobilized to a  
15 support.

131. A probe according to claim 2, wherein said at least one detectable label comprises an  
interactive label pair comprising a first label moiety conjugated to said first object sequence and  
20 a second label moiety conjugated to said first complement sequence, said first and second label  
moieties interacting when said first hybridized duplex is formed.

132. A probe according to claim 2, wherein said at least one detectable label comprises an  
interactive label pair comprising a first label moiety conjugated to said second object sequence  
25 and a second label moiety conjugated to said second complement sequence, said first and second  
label moieties interacting when said second hybridized duplex is formed.

133. A probe according to claim 2, wherein said at least one detectable label comprises two  
different interactive label pairs, a first label pair comprising a first label moiety conjugated to  
30 said first object sequence and a second label moiety conjugated to said first complement  
sequence and a second label pair comprising a third label moiety conjugated to said second

object sequence and a fourth label moiety conjugated to said second complement sequence, said first and second label moieties interacting when said first hybridized duplex is formed and said third and fourth label moieties interacting when said second hybridized duplex is formed.

5 134. A probe according to claim 133, wherein said first and third label moieties are a same moiety.

135. A probe according to any of claims 131-133, wherein said interactive label pair is a fluorescer and a quencher, wherein the interaction between said interactive label pair causes  
10 change in the fluorescence of said probe, said change in the fluorescence being selected from the group consisting of intensity decrease, lifetime decrease, polarization change, and wavelength change.

136. A probe according to any of claims 131-133, wherein said probe is a trimolecular probe  
15 consisting of a first molecule comprising said object sequence, a second molecule comprising said first complement sequence, and a third molecule comprising said second complement sequence.

137. A probe according to claim 136, wherein one of said first, second, and third molecules is  
20 immobilized to a support.

138. A probe according to any of claims 131-133, wherein said probe is a bimolecular probe consisting of a first molecule containing two of said object sequence, said first complement sequence, and said second complement sequence, and a second molecule containing a remaining  
25 sequence, wherein said two sequences contained in said first molecule are covalently linked by a loop moiety.

139. A probe according to claim 138, wherein said loop moiety connects any of 5' or 3' terminus of a first sequence to any of 5' or 3' terminus of a second sequences.

30 140. A probe according to claim 138, wherein said loop moiety is a nucleic acid sequence having

about 4 to about 100 nucleotides.

141. A probe according to claim 140, wherein a first sequence, said loop moiety, and a second sequence are covalently linked in sequence in a 5' to 3' or 3' to 5' direction.

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142. A probe according to claim 138, wherein said first or second molecules is immobilized to a support.

10 143. A probe according to any of claims 131-133, wherein said probe is a unimolecular probe, wherein said object sequence, said first complement sequence, and said second complement sequence are covalently linked by at least one loop moiety.

144. A probe according to claim 143, wherein said loop moiety is a nucleic acid sequence having about 4 to about 100 nucleotides.

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145. A probe according to claim 144, wherein said first complement sequence, a first loop moiety, said object sequence, a second loop moiety, and said second complement sequence are covalently linked in sequence in a 5' to 3' or 3' to 5' direction.

20 146. A probe according to claim 144, wherein said object sequence, a first loop moiety, said first complement sequence, a second loop moiety, and said second complement sequence are covalently linked in sequence in a 5' to 3' or 3' to 5' direction.

25 147. A probe according to claim 144, wherein said object sequence, a first loop moiety, said second complement sequence, a second loop moiety, and said first complement sequence are covalently linked in sequence in a 5' to 3' or 3' to 5' direction.

148. A probe according to claim 143 immobilized to a support.

30 149. A probe according to claim 2, wherein said probe is a unimolecular probe, wherein said object sequence, said first complement sequence, and said second complement sequence are

covalently linked by at least one loop moiety, and said at least one detectable label comprises at least one interactive label pair, each label pair comprising first and second label moieties conjugated to different positions of said unimolecular probe, said first and second label moieties interacting when said first or second hybridized duplex is formed.

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150. A probe according to claim 149, wherein at least one of said first and second label moieties is covalently linked to said loop moiety.

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151. A probe according to claim 149, wherein said interactive label pair is a fluorescer and a quencher, wherein the interaction between said interactive label pair causes change in the fluorescence of said probe, said change in the fluorescence being selected from the group consisting of intensity decrease, lifetime decrease, polarization change, and wavelength change.

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152. A probe according to claim 149 immobilized to a support.

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153. A probe according to claim 2, wherein said probe is a bimolecular probe consisting of a first molecule containing two of said object sequence, said first complement sequence, and said second complement sequence, and a second molecule containing a remaining sequence, wherein said two sequences contained in said first molecule are covalently linked by a loop moiety, and said at least one detectable label comprises at least one interactive label pair, each label pair comprising first and second label moieties conjugated to different positions of said bimolecular probe, said first and second label moieties interacting when said first or second hybridized duplex is formed.

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154. A probe according to claim 153, wherein at least one of said first and second label moieties is covalently linked to said loop moiety.

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155. A probe according to claim 153, wherein said interactive label pair is a fluorescer and a quencher, wherein the interaction between said interactive label pair causes change in the fluorescence of said probe, said change in the fluorescence being selected from the group consisting of intensity decrease, lifetime decrease, polarization change, and wavelength change.

156. A probe according to claim 153, wherein said first or second molecule is immobilized to a support.

5 157. A probe according to claim 2, wherein said probe further comprises a pair of nucleic acid arm sequences consisting of a 5' arm sequence covalently linked to 5' terminus of said first complement sequence and a 3' arm sequence covalently linked to 3' terminus of said first complement sequence, said pair of arm sequences forming a stem duplex having about 3 to about 35 complementary base pairs when said first complement sequence is not hybridized to said first  
10 object sequence; and wherein said at least one detectable label comprises an interactive label pair comprising a first label moiety conjugated to said 5' arm sequence and a second label moiety conjugated to said 3' arm sequence, said first and second label moieties interacting when said stem duplex is formed.

15 158. A probe according to claim 2, wherein said probe further comprises a pair of nucleic acid arm sequences consisting of a 5' arm sequence covalently linked to 5' terminus of said second complement sequence and a 3' arm sequence covalently linked to 3' terminus of said second complement sequence, said pair of arm sequences forming a stem duplex having about 3 to about 35 complementary base pairs when said second complement sequence is not hybridized to said  
20 second object sequence; and wherein said at least one detectable label comprises an interactive label pair comprising a first label moiety conjugated to said 5' arm sequence and a second label moiety conjugated to said 3' arm sequence, said first and second label moieties interacting when said stem duplex is formed.

25 159. A probe according to claim 2, wherein said probe further comprises two pairs of nucleic acid arm sequences, a first arm pair consisting of a first 5' arm sequence covalently linked to 5' terminus of said first complement sequence and a first 3' arm sequence covalently linked to 3' terminus of said first complement sequence and a second arm pair consisting of a second 5' arm sequence covalently linked to 5' terminus of said second complement sequence and a second 3'  
30 arm sequence covalently linked to 3' terminus of said second complement sequence, said first arm pair forming a first stem duplex having about 3 to about 35 complementary base pairs when

said first complement sequence is not hybridized to said first object sequence and said second arm pair forming a second stem duplex having about 3 to about 35 complementary base pairs when said second complement sequence is not hybridized to said second object sequence; and wherein said at least one detectable label comprises two interactive label pairs, a first label pair comprising a first label moiety conjugated to said first 5' arm sequence and a second label moiety conjugated to said first 3' arm sequence and a second label pair consisting of a third label moiety conjugated to said second 5' arm sequence and a fourth label moiety conjugated to said second 3' arm sequence, said first label pair interacting when said first stem duplex is formed and said second label pair interacting when said second stem duplex is formed.

160. A probe according to any of claims 157-159, wherein said interactive label pair is a fluorescer and a quencher, wherein the interaction between said interactive label pair causes change in the fluorescence of said probe, said change in the fluorescence being selected from the group consisting of intensity decrease, lifetime decrease, polarization change, and wavelength change.

161. A probe according to any of claims 157-159, wherein said probe is a trimolecular probe consisting of a first molecule comprising said object sequence, a second molecule comprising said first complement sequence, and a third molecule comprising said second complement sequence.

162. A probe according to claim 161, wherein one of said first, second, and third molecules is immobilized to a support.

163. A probe according to any of claims 157-159, wherein said probe is a bimolecular probe consisting of a first molecule containing two of said object sequence, said first complement sequence, and said second complement sequence, and a second molecule containing a remaining sequence, wherein said two sequences contained in said first molecule are covalently linked by a loop moiety.

164. A probe according to claim 163, wherein one of said first and second molecules is immobilized to a support.

165. A probe according to any of claims 157-159, wherein said probe is a unimolecular probe,  
5 wherein a first molecule comprising said object sequence, a second molecule comprising said first complement sequence, and a third molecule comprising said second complement sequence are covalently linked by at least one loop moiety.

166. A probe according to claim 165 immobilized to a support.

10 167. An assay for detecting in a sample the presence or absence of at least one target receptor agent that can selectively bind to a probe ligand under the conditions including a detection temperature, said assay comprising:

- 15     a) contacting said sample with a probe according to claim 5; and  
      b) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target receptor agent.

168. An assay for detecting in a sample the presence or absence of at least one target ligand under the conditions including a detection temperature, said assay comprising:

- 20     a) contacting said sample with a probe according to claim 5 in the presence of a receptor agent that can bind to both said probe and target ligands; and  
      b) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target ligand.

25 169. An assay for detecting in a sample the presence or absence of at least one target reaction inducing agent that can specifically cleave a cleavage site under the conditions including a detection temperature, said assay comprising:

- 30     a) contacting said sample with a probe according to claim 10; and  
      b) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target reaction inducing agent.



170. An assay for detecting in a sample the presence or absence of at least one target reaction inducing agent that can specifically induce a covalent coupling of a reaction site under the conditions including a detection temperature, said assay comprising:

- a) contacting said sample with a probe according to claim 27; and
- b) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target reaction inducing agent.

171. An assay for detecting in a sample the presence or absence of at least one target reaction inducing agent that can specifically convert a reaction site to a conjugation site under the conditions including a detection temperature, said assay comprising:

- a) contacting said sample with a probe according to claim 34; and
- b) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target reaction inducing agent.

172. An assay for detecting in a sample the presence or absence of at least one target reaction inducing agent that can specifically convert a reaction site to a non-conjugatable site under the conditions including a detection temperature, said assay comprising:

- a) contacting said sample with a probe according to claim 47; and
- b) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target reaction inducing agent.

173. An assay for detecting an inhibitor or enhancer for binding of a receptor agent to a probe ligand under the conditions including a detection temperature, said assay comprising:

- a) contacting a target compound with a probe according to claim 5 in the presence of said receptor agent; and
- b) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target candidate compound.

174. An assay for detecting an inhibitor or enhancer for a reaction inducing agent that can specifically cleave a cleavage site under the conditions including a detection temperature, said assay comprising:

- a) contacting a target compound with a probe according to claim 10 in the presence of said reaction inducing agent; and
- b) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target compound.

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175. An assay for detecting an inhibitor or enhancer for at least one reaction inducing agent that can specifically induce a covalent coupling of a reaction site under the conditions including a detection temperature, said assay comprising:

- a) contacting a target compound with a probe according to claim 27 in the presence of said reaction inducing agent and a destabilizing agent; and
- b) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target compound.

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176. An assay for detecting an inhibitor or enhancer for at least one reaction inducing agent that can specifically convert a reaction site to a conjugation site under the conditions including a detection temperature, said assay comprising:

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- a) contacting a target compound with a probe according to claim 34 in the presence of said reaction inducing agent and a destabilizing agent; and
- b) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target compound.

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177. An assay for detecting an inhibitor or enhancer for at least one reaction inducing agent that can specifically convert a reaction site to a non-conjugatable site under the conditions including a detection temperature, said assay comprising:

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- a) contacting a target compound with a probe according to claim 47 in the presence of said reaction inducing agent and a destabilizing agent; and
- b) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target compound.

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178. An assay according to any of claims 167-177, wherein the step of ascertaining comprises measuring the level of said characteristic signal.

179. An assay according to claim 178, wherein the step of ascertaining comprises measuring the level of said characteristic signal as a function of time.

5 180. An assay according to any of claims 167, 168, and 173 further comprising a step of contacting a target-less control with a probe according to claim 5, and a step of measuring the level of said characteristic signal at said detection temperature, wherein the step of ascertaining includes calculating a difference between the level of said characteristic signal in said control and that in said sample.

10 181. An assay according to any of claims 169 and 174 further comprising a step of contacting a target-less control with a probe according to claim 10, and a step of measuring the level of said characteristic signal at said detection temperature, wherein the step of ascertaining includes calculating a difference between the level of said characteristic signal in said control and that in  
15 said sample.

182. An assay according to any of claims 170 and 175 further comprising a step of contacting a target-less control with a probe according to claim 27, and a step of measuring the level of said characteristic signal at said detection temperature, wherein the step of ascertaining includes  
20 calculating a difference between the level of said characteristic signal in said control and that in said sample.

183. An assay according to any of claims 171 and 176 further comprising a step of contacting a target-less control with a probe according to claim 34, and a step of measuring the level of said  
25 characteristic signal at said detection temperature, wherein the step of ascertaining includes calculating a difference between the level of said characteristic signal in said control and that in said sample.

184. An assay according to any of claims 172 and 177 further comprising a step of contacting a  
30 target-less control with a probe according to claim 47, and a step of measuring the level of said characteristic signal at said detection temperature, wherein the step of ascertaining includes

calculating a difference between the level of said characteristic signal in said control and that in said sample.

5 185. An assay according to any of claims 167, 168, and 173, wherein said detection temperature is at least 5°C lower than the melting temperature of said first hybridized duplex in the absence of said receptor agent.

10 186. An assay according to any of claims 169 and 174, wherein said detection temperature is at least 5°C higher than the melting temperature of said first hybridized duplex in the absence of said reaction inducing agent.

15 187. An assay according to any of claims 170, 171, 175, and 176, wherein said detection temperature is at least 5°C lower than the melting temperature of said first hybridized duplex in the absence of said reaction inducing agent.

188. An assay according to any of claims 172 and 177, wherein said detection temperature is at least 5°C higher than the melting temperature of said first hybridized duplex in the absence of said reaction inducing agent.

20 189. An assay according to any of claims 167-177, wherein said probe is a bimolecular or trimolecular probe having at least one non-interactive label.

25 190. An assay according to claim 189, wherein said probe is not an immobilized probe and the step of ascertaining includes a step of separating hybridized and unhybridized species.

191. An assay according to claim 189, wherein said probe is an immobilized probe and the step of ascertaining includes a step of washing out unbound species.

30 192. An assay according to any of claims 167-177, wherein said detectable label is an intercalating dye.

193. An assay according to claim 192, wherein said probe is not an immobilized probe and the step of ascertaining includes a step of separating hybridized and unhybridized species.

194. An assay according to claim 192, wherein said probe is an immobilized probe and the step  
5 of ascertaining includes a step of washing out unbound species.

195. An assay according to any of claims 167-177, wherein said probe comprises at least one interactive label pair.

10 196. An assay according to 195, wherein said interactive label pair is a pair of a fluorescer and a quencher or a pair of a luminescer and a quencher.

197. An assay according to claim 195, wherein said probe is a unimolecular probe.

15 198. An assay according to claim 195, wherein said probe is a bimolecular probe.

199. An assay according to claim 195, wherein said probe is a trimolecular probe.

200. An assay according to claim 195, wherein said probe is an immobilized probe.  
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201. An assay according to any of claims 167-172, wherein said probe is a unimolecular probe comprising at least one interactive label pair consisting of a fluorescer and a quencher or a luminescer and a quencher.

25 202. An assay according to claim 201, wherein said sample is a cell or tissue, and the step of contacting includes introducing said probe inside or outside the cell or tissue.

203. An assay according to claim 173, wherein said probe is a unimolecular probe comprising at least one interactive label pair consisting of a fluorescer and a quencher or a luminescer and a  
30 quencher.

204. An assay according to claim 203, wherein said sample is a cell or tissue, and the step of contacting includes introducing said probe and said target compound inside or outside the cell or tissue.

5 205. An assay according to claim 204, wherein said receptor agent is a receptor present inside or outside the cell or tissue.

206. An assay according to claim 204, wherein said receptor agent is a receptor protein recombiantly expressed by introducing a vector encoding the receptor protein inside the cell or  
10 tissue.

207. An assay according to any of claims 174-177, wherein said probe is a unimolecular probe comprising at least one interactive label pair consisting of a fluorescer and a quencher or a luminescer and a quencher.  
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208. An assay according to claim 207, wherein said sample is a cell or tissue, and the step of contacting includes introducing said probe and said target compound inside or outside the cell or tissue.

20 209. An assay according to claim 208, wherein said reaction inducing agent is an enzyme present inside or outside the cell or tissue.

210. An assay according to claim 209, wherein said reaction inducing agent is an enzyme recombiantly expressed by introducing a vector encoding the enzyme inside the cell or tissue.  
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211. An assay for detecting in a sample the presence or absence of at least one target receptor agent that can selectively bind to a probe ligand under the conditions including a detection temperature, said assay comprising:

- 30 c) contacting said sample with a probe according to claim 58; and
- d) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target receptor agent.

212. An assay for detecting in a sample the presence or absence of at least one target ligand under the conditions including a detection temperature, said assay comprising:

- c) contacting said sample with a probe according to claim 58 in the presence of a receptor agent that can bind to both said probe and target ligands; and
- d) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target ligand.

213. An assay for detecting in a sample the presence or absence of at least one target reaction inducing agent that can specifically cleave a cleavage site under the conditions including a detection temperature, said assay comprising:

- c) contacting said sample with a probe according to claim 62; and
- d) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target reaction inducing agent.

214. An assay for detecting in a sample the presence or absence of at least one target reaction inducing agent that can specifically induce a covalent coupling of a reaction site under the conditions including a detection temperature, said assay comprising:

- c) contacting said sample with a probe according to claim 66; and
- d) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target reaction inducing agent.

215. An assay for detecting in a sample the presence or absence of at least one target reaction inducing agent that can specifically convert a reaction site to a conjugation site under the conditions including a detection temperature, said assay comprising:

- c) contacting said sample with a probe according to claim 70; and
- d) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target reaction inducing agent.

216. An assay for detecting in a sample the presence or absence of at least one target reaction inducing agent that can specifically convert a reaction site to a non-conjugatable site under the conditions including a detection temperature, said assay comprising:

- c) contacting said sample with a probe according to claim 73; and
- d) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target reaction inducing agent.

217. An assay for detecting an inhibitor or enhancer for binding of a receptor agent to a probe ligand under the conditions including a detection temperature, said assay comprising:

- c) contacting a target compound with a probe according to claim 58 in the presence of said receptor agent; and
- d) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target candidate compound.

218. An assay for detecting an inhibitor or enhancer for a reaction inducing agent that can specifically cleave a cleavage site under the conditions including a detection temperature, said assay comprising:

- c) contacting a target compound with a probe according to claim 62 in the presence of said reaction inducing agent; and
- d) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target compound.

219. An assay for detecting an inhibitor or enhancer for at least one reaction inducing agent that can specifically induce a covalent coupling of a reaction site under the conditions including a detection temperature, said assay comprising:

- c) contacting a target compound with a probe according to claim 66 in the presence of said reaction inducing agent and a destabilizing agent; and
- d) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target compound.

220. An assay for detecting an inhibitor or enhancer for at least one reaction inducing agent that



can specifically convert a reaction site to a conjugation site under the conditions including a detection temperature, said assay comprising:

- c) contacting a target compound with a probe according to claim 70 in the presence of said reaction inducing agent and a destabilizing agent; and
- d) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target compound.

221. An assay for detecting an inhibitor or enhancer for at least one reaction inducing agent that can specifically convert a reaction site to a non-conjugatable site under the conditions including a detection temperature, said assay comprising:

- c) contacting a target compound with a probe according to claim 73 in the presence of said reaction inducing agent and a destabilizing agent; and
- d) ascertaining any change in the level of said characteristic signal at said detection temperature compared to that in the absence of said target compound.

222. A kit comprising at least one probe according to any of claims 1 and 2 and instructions for performing an assay for detecting at least one target agent or target ligand, or for detecting inhibitors or enhancers that inhibit or enhance interaction of the target agent with said recognition element.

223. A kit according to claim 211 further including one or more reagents selected from the group consisting of salts, buffers, nuclease inhibitors, substrates for enzyme or enzyme-coupled labels, receptor agents, reaction-inducing agents, vectors encoding receptor proteins or enzymes that act as target agents, and coupling reagents.

224. A kit according to claim 211 further including one or more components suitable for performing an *in vivo* or *in situ* assay, wherein said component is a component necessary for introducing said probe into a cell selected from permeabilizing agents and liposome precursors.

225. A kit according to claim 211, wherein said probe is an immobilized probe.

226. A kit according to claim 214 including at least one additional immobilized probe according to this invention having a different target agent and immobilized to the same support at a predetermined location.

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227. A kit according to claim 214 including at least one additional immobilized probe according to this invention having a same target agent and immobilized to the same support at a different predetermined location.

10 228. A target detection system comprising at least one probe according to any of claims 1 and 2.